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Ph.D. FINAL THESIS SYNOPSIS

For the award of the degree of Doctor of Philosophy in Botany

ETHNOPHARMACOLOGICAL VALIDATION OF MEDICINAL PLANTS OF DAKSHINA KANNADA

By

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INTRODUCTION

People have used medicinal plants for their therapeutic benefits ever since human civilization first emerged. Approximately 80% of the world's population currently relies primarily on traditional medicines for their primary health care, showing the vital role that plant-based traditional medicine systems continue to play in healthcare (Owolabi *et al.,* 2007). Due to its great cultural and floristic diversity, India is a valuable source of information on traditional applications of plant resources. The numerous researches that have been reported on this aspect from the nation have amply demonstrated this (Bhandary and Chandrashekar, 2014; Lalramanghinglova and Jha, 1999). For the treatment of illnesses, the tribal and indigenous populations of our nation frequently use ethnomedicine, one of the medical systems. Since the beginning of time, primitive communities have relied on herbal treatments to alleviate illnesses and conditions (Singh *et al.,* 1997). The mythology and culture of India are deeply rooted in the art of herbal medicine (Harsha, 2003).

Ancient Indian Sanskrit literature such as the Rigveda (about 4000–1500 BC), Atharvaveda (1500 BC), Upanishads (1000–600 BC), Mahabharata, and Puranas (700–400 BC), among others, mention a variety of uses for plants. Sushruta Samhita (about 500 BC), Charaka Samhita (around 100 AD), and Ashtanga Hridaya Samhita (200 AD) all discuss the uses of about 1200 plant medications, as well as their actions and specialized medicinal applications. There are actually many more reports of plants, but their identities have not yet been determined (Jain, 1967).India is one of the world's key hotspots for plant diversity, home to a large range of plant species. Nevertheless, only 2.4% of the world's total land area is made up of it. With about 49,000 plant species, 4,900 of which are indigenous, the nation represents an average of 8% of the total biodiversity of the world (Kumar and Asija, 2000).

More than 10,000 kinds of wild plants, including over 8,000 species with medicinal benefits, are used by India's tribal and indigenous groups for a variety of purposes

(Pushpangadan and George, 2010). Polyherbal formulation (PHF) is the utilization of many plants in a medicinal preparation (Parashuraman *et al*., 2014). The concept of polyherbalism in this antiquated therapeutic method was emphasized in the Ayurvedic book "Sarangdhar Samhita," which was written centuries ago, about 1300 A.D. (Srivastava *et al*., 2013). In the traditional practice of Indian medicine, plant formulations and blended extracts of plants are favored above individual plants. There are many dose forms available for ayurvedic herbs, with PHF being the most common (Parasuraman *et al.*, 2010 and Jayakumar, 2010).

Plant extracts are organic substances that, depending on how phytochemicals interact, may have a positive, negative, additive, or neutral effect (Wang *et al*., 2011). Additionally, any of these effects can be produced by combining plant extracts. According to Blesson *et al*., (2015) the synergistic effect is a beneficial interaction in which the sum of the mechanisms of two or more drugs is greater than the sum of the mechanisms of the individual substances. Because they have a wide therapeutic range, are effective at low dosages and safe at high levels, and have fewer side effects when used incorrectly, polyherbal formulations are quite popular (Srivastava *et al*., 2012).

Skin disease is a common ailment and it affects all ages from the neonate to the elderly and causes different types of infection in different ways. Human skin is the outer covering layer and the largest organ in the body. It plays a vital role in defence mechanisms against infection. “Dermatitis” is a word used to describe a number of skin irritations and rashes caused by genetics, an overactive immune system, infections, allergies, irritating substances and more. Common symptoms include dry skin, redness and itchiness (Marks and Miller, 2017).

The traditional knowledge system of ethnic societies is fast eroding. There is an urgent need to inventories and record all ethno-biological information among the diverse ethnic communities before the traditional cultures are completely lost. Tribal people have

thorough knowledge of herbal medicine. But tribal are gradually losing their knowledge of herbal medicine. Their knowledge has to be safeguarded therefore a list have been prepared of plants with their medicinal value found in area (Gupta *et al.*, 2010). Following are the main objectives of this study.

AIM AND OBJECTIVES

AIM: The present research work is focused on formation, evaluation and validation of Polyherbal formulation (PHF) used for Atopic dermatitis (AD).

OBJECTIVES

1. Ethnobotanical survey of medicinal plants of Dakshina Kannada District.

2. Formation and physicochemical analysis of polyherbal formulation.

3. Phytochemical evaluation and antimicrobial susceptibility of polyherbal formulation.

4. Pharmacological validation of polyherbal formulation.

The whole work is divided into 6 chapters.

The first chapter introduces the ethnobotanical work, polyherbal formulation and dermatitis. The second chapter deals with literature review of ethnobotanical work, polyherbal formulation and dermatitis. The third chapter materials and methods involve *In vitro* and *In vivo* study of Polyherbal formulation extracts. Fourth and fifth chapter deals with results, discussion and sixth chapter includes conclusion.

CHAPTER -1: INTRODUCTION

This chapter presents the thesis' prologue, which is crucial to our investigation. The first chapter explains ethnobotanical survey, polyherbal formulation (PHF), skin diseases (Dermatitis), and pharmacological validation of polyherbal formulation. A new PHF against

atopic dermatitis was developed based on an ethnobotanical survey. The powder of a polyherbal formulation was subjected to extraction, preliminary phytochemical screening, phytochemical quantification, Gas chromatography-mass spectrometry (GC-MS) analysis, antioxidant activity, *In vitro* cytotoxicity testing, antimicrobial testing a, *In vivo* acute dermal toxicity testing, and *In vivo* anti-atopic dermatitis testing during the process of pharmacological validation.

CHAPTER -2: LITERATURE REVIEW

The relevant published literature relating to the current investigation were referred and discussed. The chapter reviewed the body of literature on ethnobotany, including both historical and contemporary studies in India and other countries (Jain, 1967; Singh *et al*., 1997; Harsha, 2003; Owolabi *et al*., 2007; Pushpangadan and George, 2010). Assessment of qualitative and quantitative ethnobotanical data (Hoffman and Gallaher,2007; Savikin *et al*., 2013) the importance of polyherbal formulation (Jayakumar,2010; Parasuraman *et al*., 2010; Wang *et al*., 2011; Srivastava *et al*., 2012; Parashuraman *et al*., 2014 and Blesson *et al*. 2015), Atopic dermatitis (Leung and Bieber, 2003 ; Marks and Miller, 2006), pre-extraction and extraction methods (Harborne, 1973; Khandelwal, 2007; Ebadi *et al*., 2015 and Ngono *et al*., 2018), Organoleptic and physicochemical evaluation (Kokate *et al*., 1994; Siddiqui, 1995; Shaikh, *et al.*, 2007; Bele and Khale, 2011), qualitative and quatitative phytochemical analysis (Harborne, 1973; Rajpal, 2002; Raaman, 2006; Cicco, *et al*., 2009; Olajire and Azeez, 2011), GC-MS analysis (Jayakar *et al*., 2020), Antioxidant activity (Achola and Munenge ,1998; Rao and Muralikrishna, 2006), Antimicrobial susceptibility (Daoud *et al*., 2015), *In vitro* Cytotoxicity study (Mosmann, 1983; Shoemaker *et al*., 2004; Peng *et al*., 2005), *In vivo* acute dermal toxicity study according to OECD 402 (Zahi *et al*., 2015; Sari *et al*., 2016), and *In vivo* Anti-atopic dermatitis activity according to OECD 429 (Trang, and Son, 2017).

CHAPTER -3: MATERIALS AND METHODS

Study Area: According to a literature review, the Dakshina Kannada district (South Canara) in Karnataka state was chosen for the ethnobotanical survey due to the paucity of previous work in the field. The district is bounded by the districts of Udupi to the north, Chikamagaluru to the northeast, Hassan to the east, Kodagu to the southeast, Kasaragod of Kerala to the south and Arabian Sea to the west, i.e. 12.8438° N, 75.2479°E.

Ethnobotanical survey method: According to the practitioner's convenience open-ended questionnaire or guided field walk approach was used during the ethnobotanical survey (Martin, 1995 and Maundu, 1995). On a walk through the forest with the healers, it was possible to confirm the medicinal plants they use for their treatments and to get thorough information. Both qualitative and quantitative methods were utilized to analyses ethnobotanical data. The plants with the greatest fidelity value (FL) were chosen for the formation of Polyherbal formulation (PHF).

During the process of pharmacological validation, the powder of a polyherbal formulation was subjected to extraction, organoleptic and physicochemical evaluation, preliminary phytochemical screening, phytochemical quantification, GC-MS analysis, antioxidant activity, *In vitro* cytotoxicity testing, antimicrobial testing, *In vivo* acute dermal toxicity testing, and *In vivo* Anti- atopic dermatitis testing. Following methods and instruments were used for above work.

1. Preparation of polyherbal formulation (PHF): Novel polyherbal formulation (PHF) was developed from nine indigenous medicinal plants selected from an ethnobotanical survey that were used to treat various skin conditions were precisely mixed according to ayurvedic principles.

2. Pre-extraction preparation of medicinal plant samples: Water was used to wash and sterilize plant samples to get rid of any contaminants like dust. After being cleaned, plant samples are dried under the shade for a week or until they are brittle. As per WHO recommendations, dry samples were individually crushed into powder using a mill. (Ebadi *et al*., 2015 and Ngono *et al*., 2018)

3. Organoleptic and physicochemical evaluation: Organoleptic testing, which evaluates formulation's colour, flavour, aroma, appearance, particle size, and texture, was done on a polyherbal formulation (Siddiqui, 1995). Additionally, physicochemical examination was carried out using a number of parameters, including total ash value, acid insoluble ash, water soluble ash, extractive value, and moisture content (Kokate *et al*., 1994; Shaikh *et al*., 2007; Bele and Khale, 2011).

4. Plant extraction: The cold maceration and successive soxhlet extraction procedures were used to extract the fine powder of the polyherbal formulation (PHF). PHF powder was extracted using water, methanol, ethyl acetate, and hexane in that order (Harborne, 1973 and Khandelwal, 2007).

5. Preliminary phytochemical screening : Using common qualitative analytical techniques, the presence of various phytochemical components, including alkaloids, sugars and glycosides, saponins, proteins and amino acids, phenolic compounds and tannins, flavonoids, and others, was determined in the extracts of a NMC (Rajpal, 2002 and Raaman, 2006).

6. Quantification of phytochemicals: The phenolic and tannin content were calculated using the Cicco *et al*. (2009) technique. Olajire and Azeez (2011) proposed a method for calculating flavonoid content. The Harborne (1973) method was used to calculate the alkaloid content.

7. Antioxidant activity: The DPPH radical scavenging activity was tested using the Rao and Muralikrishna, (2006) method. The formula below was used to calculate the antioxidant activity in percent inhibition.

$$DPPH\ scavenging\ effect\ (\%) or\ Percent\ inhibition\ =\ \ A0\ -\ A\,1\ /\ A0\ \times\ 100.$$

IC_{50} Value: The simplest method for calculating IC_{50} is to plot the data as x-y and fit it with a straight line (linear regression). Next, the IC_{50} value is estimated using the fitted line (Achola and Munenge ,1998). IC_{50} value was calculated.

8. Gas Chromatography-Mass Spectrometry (GC-MS) analysis: Identification was confirmed using molecular structure, molecular mass, and computed fragments. The National Institute of Standards and Technology (NIST) library and Wiley's standard mass spectral database were used to analyze the GC-MS results. By comparing each component's average peak area to the total area, the relative percentage quantity of each component was calculated (Jayakar *et al*., 2020).

9. Antimicrobial activity: To test the antibacterial and antifungal properties of four different solvent extracts of PHF, the agar well diffusion method was employed (Daoud *et al*., 2015). Antimicrobial activity of aqueous, methanol, ethyl acetate and hexane extracts were conducted at concentration of 1000 µg /ml (1mg/ml) against bacterial strains like *Staphylococcus aureus* (Gram +ve) and *Pseudomonas aeruginosa* (Gram -ve) and fungal species like *Candida albicans* and *Trichophyton rubrum* which causes pathogenic dermatitis. Streptomycin and Amphotericin B (10 µg /ml) were used as standard antibiotic.

10. Cytotoxic activity by MTT assay: A cytotoxicity test of metabolic efficiency based on an evaluation of mitochondrial performance is the MTT assay. It is a colorimetric assay that depends on living cells mitochondrial succinate dehydrogenase for the conversion of yellow tetrazolium bromide (MTT) to the purple formazan derivative (Shoemaker *et al*., 2004 and

Peng *et al*., 2005). According to Mosmann's guidelines (1983), the MTT test was used to determine the cytotoxicity of the extracts of NMC. The formula below was used to calculate the proportion of viable cells.

$$\% \text{ Cell viability} = (\text{Absorbance of treated}/\text{Absorbance of untreated cells}) \text{x } 100$$

11. Experimental animals approved by the animal ethical committee: In the studies on acute dermal toxicity and anti-atopic dermatitis, female wistar albino rats (200-300 gm), aged 8 to 12 weeks, were utilized. The rats were kept in well-ventilated cages (25 ± 2°C) with access to water at all times, pelletized animal feed and natural light and dark cycles. Animal Ethics Committee regulations (Registration number: SCP/IAEC/F150/P187/2021) were followed in the treatment of the animals.

12. *In vivo* acute dermal toxicity study : Aqueous and methanol extracts of NMC were applied topically to the shaved skin of back of female wistar albino rat, weighing 200 - 300 gm, in accordance with OECD 402 recommendations. The test substance was administered to rats at a dose of 2000 mg/kg body weight (Sari, *et al.,* 2016 and Zahi *et al.,* 2015).

13. *In vivo* Anti-atopic dermatitis study: According to OECD-429 recommendations, atopic dermatitis was artificially induced in female rat, weighing 300-500 gm by topical application 200 µg /mL of 0.3 % of 1-Chloro-2, 4-dinitrobenzene (DNCB) on shaved skin of back. Induced rat treated with topical application of aqueous extract (1gm/mL) of NMC on shaved skin of back. The therapeutic effect of test drug on Atopic-dermatitis(AD) like skin lesions was assessed by measuring skin severity scores and epithermal thickness by micrometry, histopathological findings for Inflammatory cells, edema, fibrosis, hyperkeratosis, ulcer or abscess (Park *et al*., 2012).

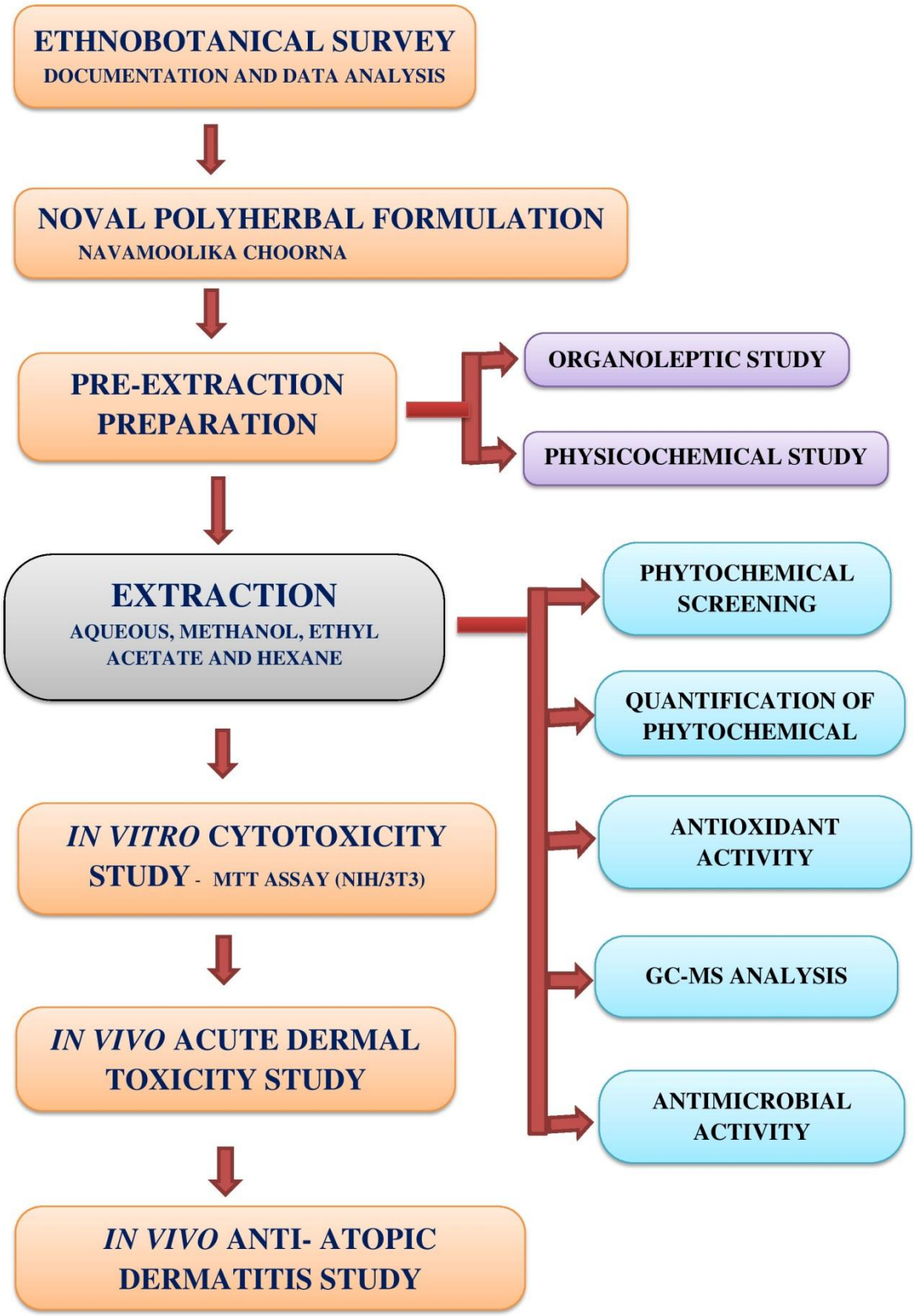

Figure 1: Flow chart of Methodology

CHAPTER - 4: RESULTS

Ethnobotanical survey: Dakshina Kannada District of Karnataka was selected for ethnobotanical study. It was possible to identify the traditional healers based on data from several sources. Interviews were conducted with 52 traditional healers who were found in Taluks throughout the district. Information about plant ingredients, parts utilized in traditional medicines, forms of medicine, methods of administration, and local names of specific plants for various illnesses has been gathered. Additionally, traditional healers' addresses, phone numbers, age, education levels, occupations, sources of ethnomedical knowledge, experiences and specializations, patient visits, information about successors, and other details were recorded.

Ethnomedicinal plants were identified using the guided field walk method, and their identification was later verified using recognized floras such as the Flora of Karnataka (1996), Flora of Udupi (2003), Flora of South Canara (2014), The plant list (2010), and also verified by Pilikula Herbarium, Pilikula Development Authority, Mangalore, D.K., and Jeddu Ayurveda and Anusandana Samsta (JASA), Alike, Bantwala, D.K.

Both qualitative and quantitative methods were utilized to analyses ethnobotanical data. Qualitative information like the botanical name, family, local name, portion used, habit, and status were gathered. Quantitative indices such as Use report (UR), Relative frequency citation (RFC), Use value (UV), Family use value (FUV), Family importance value (FIV), Cultural importance index (CI), Percentage of habit used, Consensus value for plant part (CPP), Fidelity level (FL), Informants consensus factor (ICF), and the Percentage of plant species used were used for each ethnomedicinal plants. The plants with the greatest fidelity value were chosen for the Polyherbal formulation (PHF) known as Navamoolika choorna (NMC). All nine ethnomedicinal herbs were widely utilized to treat a variety of skin ailments. As a result, the NMC was created for use in the treatment of Dermatitis.

Formation of Polyherbal Formulation (PHF): Navamoolika choorna (NMC) is a PHF made up of nine ethnomedicinal plants listed in Table -1 with their botanical names, family names, common names, parts utilized, and proportion of plant parts used.

SL NO	PLANT NAME	FAMILY	PART	PROPORTION / WEIGHT IN GRAM	
1.	*Indigofera tinctoria* L.	Leguminosae	Leaf	1 Part	10gm
2.	*Jasminum malabaricum* Wight	Oleaceae	Leaf	1 Part	10gm
3.	*Memecylon malabaricum* Cogn.	Melastomataceae.	Tender Leaf	1 Part	10gm
4.	*Breynia vitis-idaea* (Burm.f.) C.E.C.Fisch.	Phyllanthaceae	Leaf	1 Part	10gm
5.	*Vitex negundo L.*	*Lamiaceae*	Leaf	½ part	5gm
6.	*Salacia reticulata* Wight	Celastraceae	Root	½ part	5gm
7.	*Ixora coccinea* L.	Rubiaceae.	Root	½ part	5gm
8.	*Terminalia chebula* Retz.	Combretaceae	Fruit	½ part	5gm
9.	*Acacia chundra* (Rottler) Willd.	Leguminosae	Stem Bark	½ part	5gm

Table-1: Composition of Navamoolika choorna (NMC).

Organoleptic and physicochemical properties: NMC powder was military green in colour, as per Panton colour chart No. T2-GN3-T, light bitter in taste, light fruity in odour, and free of clumping and aggregation. The physicochemical screening results showed that total ash value, acid insoluble and water soluble ash values were found to be 7.70 %, 1.85 % and 3.90 % W/W respectively. The loss on drying at 105^0C was found to be 5.30 % W/W. The pH was found to be 5.70 for formulation which was very near to the neutral pH.

Extract yield percentage: The extractive values of aqueous (18.50 and 23.40%), methanol (11.70 % and 13.50 %), Hexane (6.77 % and 7.20 %) and Ethyl acetate (2.85 % and 2.33 %) in cold maceration and soxhlet extraction method respectively.

Preliminary phytochemical screening: The phytochemical analysis is critical for evaluating a plant's potential medical properties as well as determining the active principles responsible

for the plant's recognized biological activity. Aqueous solvent, extract of phytochemicals such as carbohydrates, protein, resins, phenolics, tannins, saponins and flavonoids. Methanol solvent, extract the following phytochemical such as carbohydrates, saponins, phenolics, tannins and flavonoids. Ethyl acetate solvent, extract the resins, phenolics, saponins and tannins. Hexane solvent, extract the resins, flavonoids, phenolics, tannins and alkaloids.

Quantification of phytochemical constituents: Aaqueous and methanol extracts of NMC had phenolic content of 18.98 ± 1.46 and 20.3 ± 0.96 mg/g, respectively, whereas ethyl acetate and hexane had insignificant amounts. Other bioactive compounds, including tannins and flavonoids, were identified in aqueous, methanol, ethyl acetate, and hexane extracts, although in trace quantities and could not be quantified.

GC-MS data analysis: The chromatogram of NMC extracts in aqueous, methanol, ethyl acetate, and hexane showed 13, 30, 25, and 25 peaks respectively. In aqueous extract Lup-20(29)-En-3-One (20.65 %), Stigmast-4-En-3-One (14.98 %) and 2,4-Ditert-Butylphenol (13.11 %) were the top three primary ingredients. The top three main chemicals found in the methanol extract based on abundance were alpha-methyl-D-mannopyranoside (10.5 %), hexadecanoic acid, methyl ester (10.26 %), and ethyl oleate (6.57 %). Bis (2-ethylhexyl) phthalate (34.15 %) was found in ethyl acetate crude extract, followed by sulphuric acid, dimethyl ester (13.66 %), hexadecanoic acid and methyl ester (12.62 %). The top three main components in the hexane crude extract were hexadecanoic acid, methyl ester (27.56 %), phthalic acid, di (6-methylhept-2-yl) ester (22.97 %), and methyl stearate (9.06 %).

DPPH radical scavenging activity: Antioxidants are phytochemicals that may protect cells against free radicals, which may play a role in heart disease, cancer and other diseases. At concentrations of 10 to 50 µg /mL extract, IC_{50} values of aqueous, methanolic, ethyl acetate, and hexane extracts of NMC and ascorbic acid are 26.93 µg /mL, 14.72 µg /mL, 139.86 µg

/mL, 122.23 µg /mL and 10.96 µg /mL respectively. So, at lower concentrations, methanol extract showed its highest antioxidant activity.

Antimicrobial susceptibility study (AST): The NMC extracts were tested against bacterial pathogens and fungal pathogens are an important task to determine possible drug resistance against pathogenic dermatitis under *In vitro* condition. *Staphylococcus aureus*, *Candida albicans* and *Trichophyton rubrum* were showed inhibition zone less than 05 mm diameter in all extracts and *Pseudomonas aeruginosa* was showed less than 08mm diameter inhibition zone in methanol, ethyl acetate and hexane extracts at concentration of 1000 µg/mL against standard antibiotic such as Streptomycin (10 µg/mL) and Amphotericin B (10 µg/mL). So Minimum inhibition concentration (MIC) test were not conducted for the extracts.

***In vitro* cytotoxicity study:** In drug discovery, cytotoxicity is a critical endpoint when evaluating the fate and effects of a compound. To determine the cytotoxic effect of NMC extracts by MTT assay on mouse fibroblast cell line (NIH/3T3) the viable cells were assessed in different concentrations. Dose-dependent cell viability reduction after 24hr was observed in extracts treated on NIH/3T3 cell line. The MTT assay results, expressed as IC_{50} value. The dose-dependent NIH/3T3 cell line viability was decreased after 24hr in the extracts-treated. The concentration of 500µg/mL showed more than 50% decrease in the cell viability. The IC_{50} values of cytotoxicity study showed in aqueous extract (>500 µg/mL) methanol extract (448.40 µg/mL), ethyl acetate extract (>500 µg/mL) and hexane (>500 µg/mL) of NMC stimulated the growth of the NIH/3T3 cell line. According to the result of cytotoxicity, four extract were showed nontoxic effect on NIH/3T3 cell line.

***In vivo* acute dermal toxicity Study:** In acute dermal toxicity study according to the OECD guidelines - 402, there were no signs of inflammation, no physical and behavioural changes on topical application of aqueous and methanol extracts of NMC at the concentration of 2000

mg/kg to the shaved skin of back of female albino rats, weighing 200 - 300 gm. From the study it is inferred that the drug is not toxic.

***In vivo* anti-atopic dermatitis activity Study:** According to OECD-429 recommendations, topical application of aqueous extract (1gm/mL) of NMC on shaved skin of back of DNCB(1-Chloro-2,4-dinitrobenzene) induced albino female rats, reduced the atopic dermatitis (AD) score, epidermal thickness and suppressed histopathological symptoms such as epithelium is thin stratified squamous keratinized consisting of 2 to 4 cell layers thickness, inflammatory infiltration is reduced in all areas of dermis, no ulcer, edema or fibrosis seen.

Epidermal thickness: In induced rat (141.76 µm) epidermis was very thick compared to naive rat (121.82 µm). In treated (118.9 µm) ones, epidermis restore the original thickness compared to naive rat (121.82 µm) were measured under 400x magnification by micrometry method.

CHAPTER -5: DISCUSSION

The physicochemical screening results revealed that the total ash value, acid insoluble ash value, and water soluble ash value were 7.70 %, 1.85 %, and 3.90 % W/W, respectively. Because of the presence of impurities during formulation preparation, the total ash value in the formulation is relatively high. The presence of siliceous impurities is indicated by a low acid insoluble ash value. The water soluble ash value indicated that the formulation contained fewer inorganic components such as sulphate, chlorides, sodium, and calcium (Ajazuddin, and Shailendra, 2010). The drying loss at 105^0 C was found to be 5.30 % W/W. The formulation's moisture content was within an acceptable range (5-8 %), implying that it can be stored for an extended period of time and will not be easily attacked by bacteria.

The pH of the formulation was found to be 5.70, which was very close to the neutral pH, indicating that the formulation can be used without risk of skin irritation (Khan *et al.,*

2013). In cold maceration and soxhlet extraction methods, the extractive values of aqueous extract (18.50 % and 23.40 %) and methanol extracts (11.70 % and 13.50 %) were calculated. Because the extractive value of aqueous and methanol is relatively high, it follows that water and methanol are better solvents for NMC extraction than the other solvents (Ajaz, and Shailendra, 2010). Overall, the results showed that soxhlet extraction yielded more biocompounds than cold maceration (Sankeshwari *et al.,* 2018).

The aqueous solvent extracts the most compounds, followed by methanol, ethyl acetate, and hexane. All NMC extracts contain a relatively high concentration of phenols and tannins, followed by flavanoides, saponins, and resins. The formulation is said to be high in phenolics, tannins, flavanoides, saponins, and resins. There are numerous biological benefits of phenolic compounds, including antioxidant, free radical scavenging, anti-inflammatory, and anti-carcinogenic properties (Canini *et al.*, 2007). Tannins are astringents, antioxidants, and free radical scavengers that promote wound healing and help treat peptic ulcers, while the presence of reducing sugars in these plants provides reductive properties (Rajurkar and Gaikwad, 2012). Flavonoids can also inhibit the activity of free radical-forming enzymes such as xanthine oxidase, peroxidase, and nitric oxide synthase, resulting in less oxidative damage to macromolecules (Cazarolli, 2008). Saponins have antifungal, antibacterial, antiprotozoal, and anticholesterol properties (Aslam *et al.,* 2009). Plant resins have traditionally been used to treat rheumatism, obesity, diarrhea, cancer, and other ailments (Patwari *et al.,* 2012).

The concentration of phytochemical constituents such as alkaloids, flavonoids, phenols, and tannins in polyherbal formulations has the potential to serve as a source of effective pharmaceuticals. As a result, these are necessary for good health; phytoconstituents may be a source of useful medications. Only phenols and tannins were found in moderate concentrations in aqueous and methanol extracts, but the remaining phytochemicals were

found in negligible amounts and were difficult to quantify. Methanol was found to have a higher phenolic content than aqueous extract. The results revealed that methanol and aqueous solvents extracts phenolic components from formulations better than other solvents (Dhawan and Gupta, 2016).

The most bioactive compounds were extracted using methanol solvent, according to GC-MS data analysis. Lup-20(29)-En-3-One (20.65%), Stigmast-4-En-3-One (14.98 %) and 2, 4-Ditert-Butylphenol (13.11 %) were present only in aqueous extract. There are different concentrations of hexadecanoic acid, methyl ester, in methanol (10.26 %), ethyl acetate (12.62 %), and hexane extracts (27.56 %). Only methanol (10.5 %) includes alpha-methyl-D. Bis (2-ethylhexyl) phthalate was present in large amounts (34.15 %) in the crude ethyl acetate extract, but only in very small percentages (1.6 %) and 0.72 % in the methanol and hexane extracts. Sulfuric acid dimethyl ester (13.66 %) is only present in ethyl acetate extract. Ethyl acetate (1.27 %) and hexane (22.97 %) both contain the phthalic acid di (6-methylhept-2-yl) ester. Methyl stearate is present in methanol (1.63 %), ethyl acetate (2.57 %), and hexane (9.06 %). Ethyl oleate (6.57 %) is only present in the methanol extract.

According to studies, the bioactive compounds listed above are physiologically active chemicals. According to the literature, they have pharmacologic properties, which may contribute to the plant's healing potential. Lup-20(29)-en-3-one possess different biological activities such as anti-inflammatory, anti-oxidant, wound healing and anti-cancer (skin cancer) (Gallo and Sarachine, 2009). Stigmast-4-en-3-one has antibacterial, hypoglycemic, antioxidants and anti-inflammatory activity (Yao *et al.,* 2022). 2, 4-Ditert-butylphenol possess antioxidants, anti-inflammatory, antibacterial and anticancer activity (Seenivasan *et al.,* 2022 and Murakami *et al.,* 2015). Hexadecanoic acid, methyl ester has anti-inflammatory properties (Hema *et al.,* 2011). Bis (2-ethylhexyl) phthalate is has antimicrobial properties (Lotfy *et al.,* 2018). Ehyl oleate has antibacterial properties, according to Akin-Osanaiye *et*

al. (2011). Derewenda *et al.* (1989) found that alpha.-methyl-D-mannopyranoside inhibited lectin-conjugate binding in organic synthesis; sulphuric acid dimethyl ester is used as a methylating agent (Shirley, 1966). Anti-inflammatory properties of methyl stearate have been demonstrated. Gastrin inhibition, antihelmintic activity, GABA aminotransferase inhibition, lipid metabolism regulation, and antihelmintic activity (Adnan *et al.*, 2019). The di (6-methylhept-2-yl) ester of phthalic acid has anti-inflammatory and antibacterial properties (Osuntokun and Ogunleye, 2017). The majority of the bioactive compounds discovered had significant biological activity, such as anti-inflammatory, indicating that Navamoolika choorna has anti-inflammatory properties.

Because the extracts were rich in Phenolics and tannins, the aqueous extract had the highest radical scavenging activity with 77.40 % at concentrations ranging from 10 to 50 μg/mL extract, followed by methanol extract with 70.85 % and ethyl acetate extract with 17.03 %. However, due to the low concentration of phenolics and tannins in hexane extract, it had very little scavenging action at high concentrations (100 μg/mL). As a result, the aqueous extract is weaker than the ascorbic acid extract but stronger than the methanol extract. The IC_{50} values showed that methanol extract inhibited at a lower concentration (14.72 μg/mL) than aqueous extract (26.93 μg/mL), but it was less potent than ascorbic acid (10.96 μg/mL) (Dhawan and Gupta, 2016).

In terms of antimicrobial activity, aqueous, methanol, ethyl acetate, and hexane extracts did not show a noticeable inhibition zone (less than 05 mm) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Trichophyton rubrum*, which may be due to the null effect (functional incompatibility) of herbs in PHF (Pole, 2013). As a result, NMC extracts were intended to be tested against Atopic dermatitis (AD).

In an *in vivo* cytotoxicity study, aqueous and methanol extracts of NMC were found to be safe. On low concentrations, extracts were non-toxic to mouse embryonic fibroblast cell lines (NIH/3T3 cells) (Sandra *et al.,* 2017).

Aqueous and methanol extracts were used in an *in vivo* acute dermal toxicity study according to OECD - 402 guidelines. The results showed that there were no signs of inflammation and no physical or behavioral changes after topical application of extracts for 14 days in both trial groups, but when compared, aqueous extract performed better than methanol extract (Sari *et al.,* 2016).

In an *in vivo* anti-atopic dermatitis activity study, an aqueous extract was topically applied to the back of a DNCB(1-Chloro-2,4-dinitrobenzene) induced albino rat, and it reduced the atopic dermatitis (AD) score, epidermal thickness, and suppressed histopathological symptoms, ultimately restoring the rat's original health condition. Hyemin Gu *et al.* (2018).

CHAPTER -6: CONCLUSION

The Dakshina Kannada district is known for its traditional treatment of a wide range of ailments, including neurological disorder, cardiovascular disorder, menstrual disorder, immunological disorder, ophthalmic disorder, osteomuscular disorder, gastrointestinal disorder, skin diseases, urogenital disorder and respiratory disorder. Traditional medicinal knowledge deteriorates year after year due to a lack of documentation.

A total of 51 traditional healers were interviewed and documented 290 ethnomedicinal plants from 82 families and 240 genera. According to ethno botanical research, the majority of traditional healers treat various skin diseases in a variety of ways using ethno medicinal plants. Based on fidelity value (FL), nine ethnomedicinal plants were chosen and developed into Navamoolika choorna (NMC), a new polyherbal formulation

(PHF). A review of the literature revealed that individual plants were effective against dermatitis. Following that, NMC was subjected to a series of studies, including analyses of its organoleptic, physicochemical, qualitative, and quantitative phytochemical components, including GCMS, as well as antioxidant activity, antimicrobial susceptibility, *In vitro* cytotoxicity, *in vivo* acute dermal toxicity, and *in vivo* anti-atopic dermatitis properties. NMC is a nontoxic polyherbal formulation, according to *In vitro* cytotoxicity and *in vivo* acute dermal toxicity studies (PHF). Both aqueous and methanol extracts were found to be safe and to have significant antioxidant activity, though they were least effective against pathogenic dermatitis. Finally, aqueous extracts proved to be the most effective in treating atopic dermatitis. More research is needed to preserve the most ethnomedicinal knowledge of the Dakshinakannada district, in addition to NMC should fractionate, purify, identify, and test for oral administration.

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1. Acharya, Manohara., Divakar, M. S., Malabadi, B. Ravindra., Chalannavar, R. K.(2022). **Ethnobotanical survey of medicinal plants used by the "Nalike community" in the Bantwala taluk of Dakshina Kannada district, Karnataka, India.** *Plant Science Today*, 9(2): 461–468.

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2. Acharya, Manohara., Divakar, M. S., Malabadi, B. Ravindra., Chalannavar, R. K., Kuladeep, P. P., Kiran, P. Kolkar.(2023). **Exploration of Ethnomedical Knowledge of "Gowda community" of Sullia Taluk, Dakshina Kkannada District, Karnataka, India**. *Biomedicine,* 43(1): 130-139.

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1. Acharya, Manohara., Chalannavar, R.K. (2022). **Ethnomedicinal plants used in treatment of skin diseases in Dakshina Kannada District, Karnataka, India**. *Frontiers in Life Science*, 1: 53-64. Kolhapur, Bhumi Publishing, ISBN: 978-81-951982-1-4.

2. Acharya, Manohara., Malabadi, B. Ravindra., Chalannavar, R. K.(2022). **Abating ethno medicinal knowledge.** *Frontiers in Life Science*, 4: 74-78. Kolhapur, Bhumi Publishing, ISBN: 978-93-91768-01-0.

PAPER PRESENTED

1. Participated and presented orally on **"Ethnomedicinal plants used by vasu pandith - a traditional healer of bantwala taluk"** in the National interaction meeting of practitioners and researchers of traditional system of medicine held on 12th -13th October 2017 at Kerala Forest Research Institute, Peechi, Trishur, Kerala.

2. Participated and presented orally on **"Documentation of Ethnomedicinal Plants Used by "Nalike" Community for Paralysis in Bantwala Taluk, D.K.**" in International conference on medicinal plants and drug discovery held on 18th -20th July 2018 at Bangalore.

3. Participated and presented orally on **"Deteriation of Trditional medicinal knowledge of Dakshina Knnada District"** held on 15th -17th September 2021 at 16th Kannda Vijnana Sammelana, Mangalore University, Mangalagangothri.

Signature of the Candidate **Signature of the Research Guide**